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IN – SILICO ANALYSIS OF NON- SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISMS OF ACTIVATION- INDUCED CYTIDINE DEAMINASE *(AICDA)* GENE

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ABSTRACT

Introduction: The *activation- induced cytidine deaminase (AICDA)* enzyme is responsible for converting the immunoglobulin IgM to other classes (IgG, IgA and IgE).Immunodeficiency type2 with hyper serum IgM (HIGM2) is due to failure of *AICDA* gene to convert the IgM to other classes. The aim of this study was to investigate the effect of nsSNPs in the function and stability of *(AICDA*) gene using computational tools. **Methods:** Various software were used to investigates the functional effect of ns SNPs of *AICDA* gene. Interactions of this gene with other genes was investigated using Gene MANIA. SNPs in the gene were obtained from dbSNP database. For functional analysis various softwares were used including SIFT, Polyphen-2, PROVEAN and SNPs &GO and PHD-SNP. In SIFT the amino acid substitution is predicted deleterious if the score is ≤0.05, and tolerated if the score is >0.05. Regarding polyphen-2 the outcome is shown as probably damaging (score=1) (more confident) possibly damaging(less confident) and PROVEAN prediction, the PROVEAN threshold is (-2.5) if the protein variant is ≤ -2.5 predicted to have a "deleterious" effect. If the PROVEAN score is above the threshold, the variant is predicted to have a "neutral" effect. Also to investigate the disease related SNPs (SNPs & GO and (PhD-SNP) were used. The variation can be classified disease-related or neutral. The stability effect of the SNPs on the protein was investigated using I-mutant suite version3.0, the reliability index:0-10,where 0 is lowest reliability and 10 is the highest. The out put is either increase or decrease stability. Lastly Project hope was used to investigate the effect of amino acids substitution in the protein structure and function. **Results:** Gene interaction using GeneMANIA showed that *AICDA* gene has an interaction with 20 other genes mainly two genes: namely *SPTY2D1, and STAT4*. SIFT predication revealed 200 nsSNPs in the gene. A total of 131 were in the coding region,61 were in 3´ UTR,and 8 were in 5´UTR. Out of these, 10 SNPs were determined to deleterious.Polyphen-2 predicted that the 10 nsSNPs were probably damaging and with high score (≥ 0.9). Likewise PROVEAN showed that all 10 SNPs were deleterious. To predict the association with disease using SNPs&GO and PHD-SNP, the former showed that 6 mutations were disease related while 4 mutations were neutral while the latter showed that 9 mutations were disease related while 1 (one) mutation was neutral. Prediction of protein stability using I-Mutant3.0 revealed that 9 SNPs lead to decrease protein stability while one mutation showed an increased stability effect. The change in the chemical nature of amino acid and how it effects the protein structure was analyzed using Project Hope. Overall five highly deleterious and damaging mutations were predicted namely, R24W (rs104894324), W80R (rs104894320), L106P (rs104894321) M139T(rs200858797) F151S (rs104894327). **Conclusion:** This study showed, after using various computer software, five highly deleterious and damaging SNPs in *AICDA* gene. These SNPs can be considered as candidate SNPs in people with HIGM2 after further conformation using laboratory techniques.

KEYWORDS: *AICDA*, computational analysis, HIGM2, SNPs.

ABBREVIATIONS

nsSNP: non synonymous single nucleotide polymorphisms, *AICDA* : *activation- induced cytidine deaminase*, HIGM2: immunodeficiency with hyper IgM type2, SNPs: single nucleotide polymorphisms, *AID*: *activation- induced cytidine deaminase* short name, rs:

reference SNPS, SIFT: sorting intolerant from tolerant, PHD-SNP: Predictor of Human Deleterious Single Nucleotide Polymorphism, SNP&GO: Single nucleotide polymorphisms and gene ontology *SPTY2D1*: *chromatin protein domain containing. 1STAT4*: *signal transducer and activator of transcription.* Indel: insertion deletion, aa: amino acid, Cds: Coding Sequence. UTR: un-translated region.

INTRODUCTION

Immunodeficiency is a state in which the ability of the immune system to fight infectious diseases or cancer is compromised or absent.[1] Immunodeficiency syndrome with hyper IgM are classified into five types. Type 2 immunodeficiency syndrome is characterized by normal or elevated serum IgM levels with low level or absence of IgG, IgA, IgE. Type 2 is inherited in an autosomal recessive manner. It is mainly caused by mutations in the gene *activation- induced cytidine deaminase (AICDA)*, short name*(AID*).[2-6] This results in failure in conversion of the IgM to other classes (IgG, IgA, IgE).[2,3]

A single nucleotide polymorphism (SNP) is a substitution of a single nucleotide that occurs at specific position in the genome. They may occur in coding and /or non coding regions. SNPs in the coding region are of two types synonymous and non synonymous SNPs. The non synonymous SNPs (which may be missense or nonsense) may affect the final protein sequence, while synonymous SNPs do not change the protein sequence.[7,8]

A variety of missenese, nonsense, small insertions or deletions, and splice variant have been described throughout the *AICDA* gene, as well as partial and whole gene deletions.[9,10]

Mutations that can be predicted using bioinformatics tools,is the science of health information. It is applying of informatics methodology to analyze the huge amount of biomedical and genetics data that is being revealed each day with the aim of generating clinical knowledge, finding similarities in general populations, suggesting points for therapy treatments.[11-14]

The aim of this study was to investigate the effect of non synonymous SNPs found in the *activation- induced cytidine deaminase (AICDA*) gene on protein function and structure using bioinformatics tools (in-silico approach).

MATERIALS AND METHODS

The information of *AICDA* gene was obtained from National Center of Biotechnology (NCBI) SNPs database (*https://www.ncbi.nlm.nih.gov/.)* in May 2019. The protein sequences were obtained form Expasy. The SNPs were analyzed using various computational softwares.

i) Interactions with other genes

One software was used GeneMANIA. It is an online software used for prediction of the relation between a set of input gene and other genes using a very large functional association data. Association data include protein and genetic interactions, pathways, co-expression, co-localization and protein domain similarity and co-expression.[15] Available at *genemania.org.*

ii) Functional analysis

a-**Sorting Intolerant from Tolerant (SIFT):** It is online bioinformatics software used to detect deleterious nsSNP[16], The downloaded rsSNPs (obtained from dbSNP database) were submitted into the software.The software searches for similar sequences,[17] close related sequences that may share similar sequences to query sequences.[18,19] It then calculates normalized probability for all possible substitutions from the alignment. Probability less than 0.05 is predicted to be deleterious, while greater than or equal to0.05 is predicted to be tolerated.[20] This software is available at:*siftdna.org/*

b-(Polymorphism phenotyping-v2(Polyphen-2): It is an online software available at *genetics.bwh.harvard. edu/pph2/.* Protein or SNP identifier or protein sequence in FASTA format and selecting the position and substitution of wild type and mutant type were the input. The software predicts the possible impact of amino acid substitutions on the stability and function of human proteins using structural and comparative considerations, phylogenetics, this program searches for 3D protein structural, multiple alignments of homology sequences and amino acid contact information in several protein structural databases then calculate position specific independent count scores (PSIC) for each of two variants, and then computes the PSIC scores difference, the higher scores were probably damaging (2.00 or more), possibly damaging(1.40-1.90), potentially damaging (1.0-1.50), benign (0.00-0.90).[21-23]

c-Protein Variation Effect Analyzer (PROVEAN): This is online bioinformatics software tool which predicts whether an amino acid substitution or indel has an impact on the biological function of protein. PROVEAN is useful for filtering sequence variants to identify nosynonymous or indel variants that predict to be functionally important, based on alignment by measuring the change in sequence similarity to query sequence before and after the mutation. The performance of PROVEAN is comparable to popular tools such as SIFT or polyphen-2,[24] The input is protein ID and mutations position, and the out put is expressed as deleterious or benign If the PROVEAN score is equal to or below a predefined threshold (-2.5), the protein variant is predicted to have a "deleterious" effect. If the PROVEAN score is above the threshold, the variant is predicted to have a "neutral" effect. This software is available at *provean.jcvi.org*

d) Disease related software

a) Single nucleotide polymorphisms and Gene Ontology (SNPs&GO): It is a web server for the prediction of human disease-related single point mutations. It is a support vector machine (SVM) based classifier.[25] The input is the protein sequence and point of mutation and the wild and mutated amino acids. The SNPs are then classified disease-related or neutral. It is available at:*snps.biofold.org/snps-and-go/snps-and-go.html.*

b) Predictor of Human Deleterious Single Nucleotide Polymorphism (PhD-SNP)

It is an online support vector machine (SVM) based classifier, by using protein sequence and mutations position to predict the effect of single point protein mutation, it can be classified as disease-related or as neutral polymorphism[26] Available at: http: //*snps.biofold.org/phd-snp/phd-snp.html.*

iii) Stability effect. One software was used namely I-Mutant suite version3.0**:** It is an online support vector machine tool based on the pro therm database to evaluate nsSNPs induced changes in protein stability starting from the protein structure or from the protein sequences I-Mutant correctly predicts whether the mutation stabilities (S),or destabilizes (D). Also it estimates the free energy change value (DDG) by calculating the unfolding Gibbs free energy value (DG) for the wild type protein and subtracting it from that of the mutant protein. It also predicts the sign (increase or decrease) of the free energy change value (DDG), along with a reliability index for the results (R1:0-10,where 0 is the lowest reliability and 10 is the highest reliability).[27] The input was the protein sequence and mutations position and the out put was a decrease or increase stability. It is available at *gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I.).*

iv) Protein modeling: Project hope. It is an online web server that collects structural information from a series of sources, including calculations on the 3D protein structure, sequence annotations in Uniprot and predictions from DAS-servers. The input method of project Hope carries the protein sequence and selection of mutant variants, the server predicts the output in the form of structural variation between mutant and wild type residues.[28] (available at*:www.cmbi.ru.nl/hope*).

RESULTS

The aim of this study was to investigate the effect of non synonymous SNPs in the *activation- induced cytidine deamainase (AICDA*) gene on the protein function and structure. The SNPs of *AICDA* gene were first download from SNP database.

To investigate the relation of *AICDA* gene with other genes, GeneMANIA results showed the interaction of the *AICDA* gene with 20 other genes. The interactions includes functional interaction, physical interaction, co-expression, co-location and pathway, *AICDA* gene has a high functional interaction with two genes that have same function as well as *AICDA* gene, they are *SPTY2D1*gene (chromatin protein domine1) and *STAT4* gene (single transducer and activation of transcription(Fig.1).

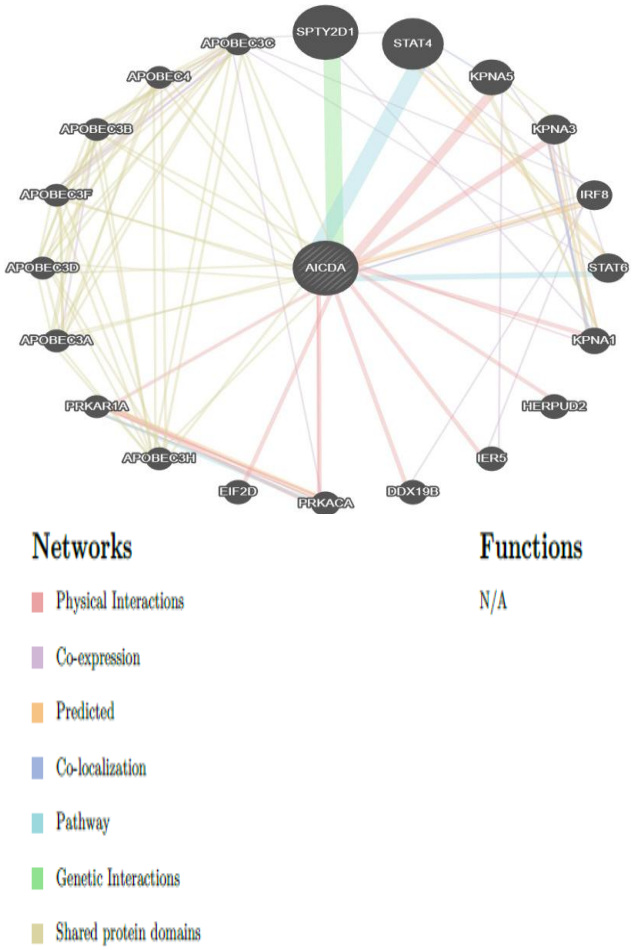


Figure 1: Ineraction of *AICDA* gene with other genes using Gene MANIA.

Functional analysis was carried out using five different softwares. The downloaded rsSNPs were submitted to SIFT software and the results showed a total of 200 SNPs present, 131 were in coding region (CDS),61 were in 3 U´TR,and 8 were in 5´ UTR.

Some of these SNPs in coding region were duplicated and thus after remove of duplications, 10 SNPS were predicted to be deleterious. (Table 1). Like wise Polyphen-2 precition revealed that all the 10 SNPs were probably damaging and with high score≥0.9 (Table 1). Also Provean prediction showed that all SNPs were deleterious (Table 1).

The association of nsSNPs to disease using SNP&GO and PHD-SNP software revealed that six SNPs out of the ten SNPs that were previously predicted to deleterious by the above software are related to disease while only four SNPs were neutral using SNPs & GO. Table [1] On the

other hand PHD-SNP predicted that 10 mutations, 9 mutations are related to disease while 1 (one) mutation is neutral. (Table.1).

Table 1: Prediction of nsSNPs using various softwares.

SNP ID	Amino acid change	Protein ID	SIFT		Polyphen		Provean		SNP&GO		PHD-SNP	
			Score	prediction	Predication	Score	predication	Score	Predication	RI	predication	RI
rs104894320	W80R	ENSP00000445691	0.017	deleterious	probably damaging	1	deleterious	-8.38	disease	6	disease	8
rs104894321	L106P	ENSP00000229335	0	deleterious	probably damaging	1	deleterious	-6.88	disease	6	disease	8
rs104894322	M139V	ENSP00000445691	0	deleterious	probably damaging	0.99	deleterious	-3.68	disease	8	disease	9
rs104894324	R24W	ENSP00000229335	0.001	deleterious	probably damaging	1	deleterious	-5.61	disease	6	disease	9
rs104894327	F151S	ENSP00000229335	0	deleterious	probably damaging	0.99	deleterious	-6.41	disease	8	disease	9
rs193922704	G125E	ENSP00000229335	0.002	deleterious	probably damaging	1	deleterious	-6.99	neutral	4	disease	1
rs199697153	Y28H	ENSP00000229335	0	deleterious	probably damaging	1	deleterious	-4.62	neutral	7	disease	1
rs200858797	M139T	ENSP00000229335	0	deleterious	probably damaging	0.994	deleterious	-5.43	disease	9	disease	9
rs375667331	T140I	ENSP00000445691	0.01	deleterious	probably damaging	0.993	deleterious	-5.01	neutral	2	disease	1
rs375684823	T140A	ENSP00000445691	0.032	deleterious	probably damaging	0.911	deleterious	-3.47	neutral	6	neutral	2

Prediction of the effect of the protein stability was done using I-Mutant3.0 server. The 10 nsSNPs that were predicted to deleterious and probably damaging by SIFT and POLYPHEN-2 soft wares, were submitted to the I-Mutant3.0 server. The results showed that the stability was decreased in 9 SNPs and increased in one (Table 2).

Table 2: Prediction of Stability using I –mutant.*

SNP ID	Amino acid change	Protein ID	I-MUTANT stability	Reliability Index
rs104894320	W80R	ENSP00000445691	Decrease	4
rs104894321	L106P	ENSP00000229335	Decrease	4
rs104894322	M139V	ENSP00000445691	Increase	1
rs104894324	R24W	ENSP00000229335	Decrease	6
rs104894327	F151S	ENSP00000229335	Decrease	9
rs193922704	G125E	ENSP00000229335	Decrease	6
rs199697153	Y28H	ENSP00000229335	Decrease	9
rs200858797	M139T	ENSP00000229335	Decrease	7
rs375667331	T140I	ENSP00000445691	Decrease	7
rs375684823	T140A	ENSP00000445691	Decrease	9

* I-Mutant: neutral mutation classification: 0.5=<DDG =<0.5,while changes<-0.5 are classified as large decrease and changes>0.5 are classified as large increase and the reliability index:0-10,where 0 is lowest reliability and 10 is highest reliability.

The predication of the effect of the substitution of the amino acids in protein structure and function was carried out using Project hope software. Five highly deleterious and damaging nonsynonymous SNPs predicted using previous softwares were submitted to project Hope software. The wild type and mutant type structures, 3Dstructure are listed in Table[3].

R24W (rs104894324), the amino acid arginine changed to tryptophan, The mutant residue is smaller than the wild-type residue. The wild-type residue charge was neutral, the mutant residue charge is positive. The wild-type residue is more hydrophobic than the mutant residue.

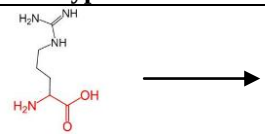
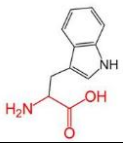
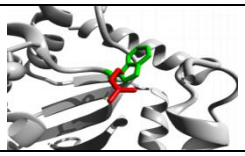
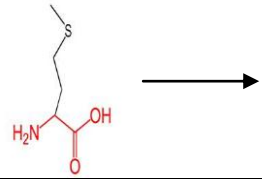
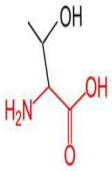
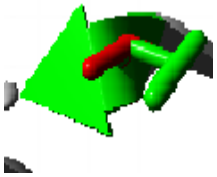
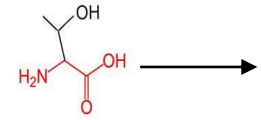
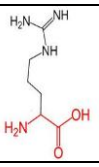
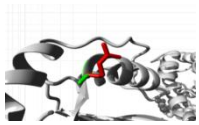
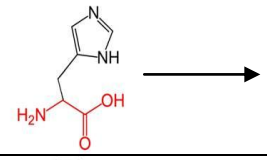
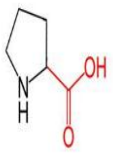
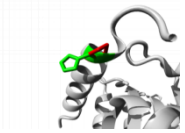
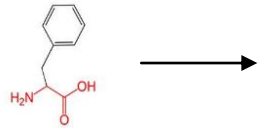
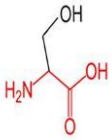
W80R (rs104894320), the amino acid tryptophan changed to arginine, The mutant residue is bigger than the wild-type residue. The wild-type residue charge was neutraL, the mutant residue charge is positive. The wild-type residue is more hydrophobic than the mutant residue.

L106P (rs104894321), the amino acid leucine changed to proline, The mutant residue is smaller than the wild-type residue. The mutant residue is more hydrophobic than the wild-type residue. And charge was lost.

M139T(rs200858797), the amino acid methionine changed to threonine, The mutant residue is smaller than the wild-type residue. The wild-type residue is more hydrophobic than the mutant residue.the charge was lost.

F151S (rs104894327) the amino acid phenylalanine changed to serine, The mutant residue is smaller than the wild-type residue. The wild-type residue is more hydrophobic than the mutant residue and the charge was lost.

Table 3: Project hope result, shown amino acids changes, the wild type, mutant type, 3Dstructure (green wild type, red mutant type).

SNP ID	AA change	Wild type	Mutant type	3Dstructure
rs104894324	R24W			
rs200858797	M139T			
rs104894320	W80R			
rs104894321	L106P			
rs104894327	F151S			

DISCUSSION

Inherited HIGM syndromes is results from the lack or decrease of the cytidine deaminase activity which can be as a result of mutations in the *ACIDA* gene. In the current computational study the aim was to investigate the nsSNPs in the *ACIDA* gene. The results revealed 10 damaging and deleterious mutations, five were highly deleterious and damaging mutations, namely: R24W (rs104894324), W80R (rs104894320), L106P (rs104894321), M139T (rs200858797), F151S (rs104894327). Six mutations have been reported in previous study carried out in Paris.[28] They were (rs104894320) W80R, (rs104894321) L106P, (rs104894322) M139V, (rs104894324) R24W, (rs104894327) F151S, (rs200858797) M139T. The other four mutations were not reported in the previous studies, they are (rs193922704) G125E, (rs199697153)Y28H, (rs375667331)T140I, (rs375684823) T140A.

Several previous studies reported mutations related to *AICDA* Gene in different countries, France Latin America Brazil, Mexico, Costa Rica, Chile and Argentina. Mutations in France are DEL6AA+F15X, R24W, DEL3AA+L59F, W68X, W80R, L106P, M139V,C147X,F151S[29] and mutation in Latin America is C87S.[30]

Another study was conducted in Brazilian populations, a new recessive mutation was found in two Brazilian sisters[31,32] with HIGM2, whose parents are first –degree cousins, the mutation is single non-silencing C to G transversion in the third position at codon15 (F15L), the mutation is homozygous in both patients and this is the first description of the F15L in *AICDA* mutation.[33,34] Another study found an arg112cys mutation in two patients in French-Canadians,[35] and two missense mutation of *AICDA* cysB7Arg and leu 98 Arg.[36] Various mutation were also reported among French population in *AICDA*.[37-40]

Furthermore another study in Finland identified a rare, homozygous variant (M 139 T) in the *AICDA* gene in four affected individuals from Eastern Finland,[41-44] this mutation also found in three Turkish patients with HIGM2.[3,45]

CONCLUSION

The results of this study showed five damaging nsSNPs in the *AICDA* gene. The application of computational tools like SIFT, Polyphen-2, Project Hope, and PROVEAN may provide an alternative approach for selecting target nsSNPs for diagnostic purposes.

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